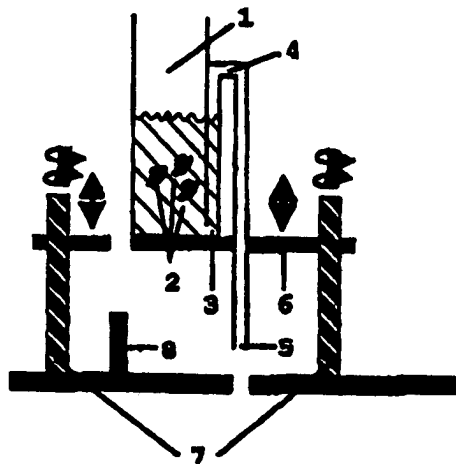




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12M 1/00, C02F 1/28 // B03C 1/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/13051</b> <b>(43) International Publication Date:</b> 18 March 1999 (18.03.99)
<b>(21) International Application Number:</b> PCT/NO98/00271 <b>(22) International Filing Date:</b> 7 September 1998 (07.09.98) <b>(30) Priority Data:</b> 974113 8 September 1997 (08.09.97) NO <b>(71)(72) Applicant and Inventor:</b> RUDI, Knut [NO/NO]; Edv. Munchs vei 83, N-1063 Oslo (NO). <b>(74) Agent:</b> ONSAGERS PATENTKONTOR – DEFENSOR AS; P.O. Box 265 Sentrum, N-0103 Oslo (NO).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> DEVICE FOR PURIFICATION OF ORGANIC MATERIAL BY ADSORBING MATERIAL TO BEADS AND ATTRACTING BEADS WITH MAGNETICAL, ELECTRICAL OR GRAVITATIONAL MEANS		
<b>(57) Abstract</b>  A device is described for purifying organic material which is immobilised and retained on solid phases. The device is characterized by that liquid is flowing through a container with separate inlet and outlet. The solid phase is retained either by physical coupling to the container, by magnetical, electrical or gravititional forces. A method for purification is also described.		



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**DEVICE FOR PURIFICATION OF ORGANIC MATERIAL BY ADSORBING MATERIAL TO BEADS AND ATTRACTING BEADS WITH MAGNETICAL, ELECTRICAL OR GRAVITATIONAL MEANS**

The present invention relates to a device for purification of organic material which is immobilised and retained on solid phases in a liquid solution. The device is based on that the liquid flows through a container via separated  
5 inlet and outlet. The solid phase is retained either by physical coupling to the container or by magnetic, electric or gravitational forces.

**Prior art**

Immobilisation of organic material on solid phases for purification is a known principle (international application no. PCT/GB91/00212). The  
10 purification strategies according to prior art are either based on that the liquid is sucked out with the same means with which it is supplied to the container, or the filtration of the liquid from the solid phase (products supplied by QIAGEN GmbH, Germany). The problem with using the same  
15 means for supplying the contaminated liquid to the purification container and the removal of the liquid after purification is that it is complicated and the risk for contamination is clearly increased. To solve this problem by  
filtration complicates the whole process, since the filtrated particle has to be removed from the filter which tends to be clogged, or the organic material  
has to be eluted from the particles.

20 In Norwegian patent application no 934915 it is described a device for removal of organic waste material from water through microbial growth on particles, with subsequent removal of these particles. This device describes however a device for purification of water only and cannot be used if the  
25 object is to purify organic material such as bacteria, nucleic acids, proteins and polysaccharides.

Thus the object of the present invention is to provide a device for purifying organic material, such as for example DNA, RNA or proteins.

This object is obtained by the present invention characterised by the enclosed claims.

30 There is presently a considerable need for purifying organic material, especially in many applications, as for example polymerase chain reaction (PCR) it is of great importance to prevent cross contamination between different samples.

The device according to the present invention is based on that the liquid used for purification is flowing in one direction only. It is never physical contact between the sample and that part of the device used for supplying the liquid. This prevents cross contamination between different samples.

- 5 The invention will in the following be explained in greater detail and by reference to figure 1.

Fig. 1a - 1d are cross sections of the device according to the invention showing the design and the different steps in the purification process.

- 10 The device according to the invention is illustrated in figure 1. It comprises a container 1 with an outlet opening 3 in the bottom of the container 1 connected to a tube with a discharge leg ascending on the outside of the container up to below the rim of the container, a U-bend 4 and a suction leg 5 ending below the bottom of the container. The container is mounted on a vertically moveable platform 6 guided by two guide bars 7 mounted on a
- 15 basis which in addition contains an attracting means 8; mounted such that when the container is in its lowest position the attracting means is closely adjacent to the container. The platform 6 has two throughgoing holes for the section leg 5 of the outlet tube and the attracting means 8. The basis has one throughgoing hole for the suction leg of the outlet tube 5, corresponding to
- 20 the same hole in the platform 6. The attracting means is a magnet or an electromagnetic device. The electromagnetic device is then activated by suitable activating means, such as a circuit switch for activation of electrical energy. The organic material to be purified can be in solution or in solid state and is added to the container together with the solid phase comprising
- 25 particles or beads or paramagnetic beads, or the beads are added to the container before the organic material to be purified, or after the organic material is added to the container. Suitable liquid is then added to the container. To rinse out all material except the material of interest which is adsorbed to the solid phase, the level of the rinsing liquid is raised to reach the
- 30 level corresponding to the U-bend 4 of the outlet tube, and the liquid will be sucked out of the container by gravitational forces until the container is empty. The flow of liquid through the container 1 may also be powered by pumps. The complexity of the device can be increased by mounting several containers together which can be moved vertically in relation to each other.

The magnetic means 8 will attract the solid phase when the container is moved to the lowest position (fig. 1b). If the liquid is added to the container in this position it will when reaching the level corresponding to the U-bend flow out of the container while the particles will be kept in position by the magnetic forces. When the container is in the upper position the solid phase will not be affected by the magnetic forces, and by moving the container slightly up and down the mixture of particles and liquid will be facilitated.

This device can for example be used for purification of bacteria and then the same solid phase can be used for purifying the DNA from this bacteria. In the upper position (fig. 1 a) the bacteria are mixed with a solution with paramagnetic particles and the bacteria will adhere to the solid phase. The container will then be lowered (fig. 1b) such that the solid phase with the bacteria adsorbed will be pulled towards the magnets. Liquid will continuously be added to the container such that the critical liquid level for emptying the container is maintained. When the bacteria have obtained the desired purity the liquid is emptied out the container (fig 1c,d) and the container is lifted to the upper position. A new liquid for rinsing of bacterial debris from the DNA and binding the DNA to the solid phase is added, and mixing of the solution by moving the container up and down in the upper position is performed. The container is then moved to the lowest position and the liquid level is kept on the critical level for emptying the container. This treatment is repeated until the DNA has reached the desired purity. In many occasions it will not be necessary to remove the organic material or DNA from the beads since for example DNA associated with the beads can be used directly in enzymatic reactions, such as the polymerase chain reaction (PCR).

For example the purified DNA can be transferred to a new container, or the original container (1) can be used for downstream applications such as thermocycling in PCR.

The device according to the invention may have several embodiments:

1. All known beads for immobilising organic molecules can be used.
2. Liquid flow can be regulated by a pump.
3. The solid phase with the organic material can be retained either by being physically connected to a container, or by gravitational forces or electric forces.
4. The liquid volumes can be varied.

5. The number of containers used in the process at the same time can vary.
6. Mechanical movements and addition of liquids can be automatized.

## CLAIMS

1. Device for purification of organic material, included biological materials, preferably in solution, comprising a container (1) for the solution, wherein the container (1) is supplied with controllable liquid inlet and outlet  
5 located at a distance from each other and which allow flow of the liquid through the container,  
c h a r a c t e r i z e d i n that the container (1) contains particles or beads for adsorbing or binding the organic material, and that the device further is equipped with means for attracting the particles, and further comprise means  
10 for activation of the attracting means for attracting the particles and thereby attracting the adsorbed or attached organic material.
2. Device according to claim 1,  
c h a r a c t e r i z e d i n that it further comprises means for controlling the flow of liquid through the container (1).
- 15 3. Device according to claim 2,  
c h a r a c t e r i z e d i n that the means for controlling the flow of liquid includes a pump.
4. Device according to one of the preceding claims,  
c h a r a c t e r i z e d i n that the outlet includes an opening (3) in the  
20 bottom of the container and a tube connected to the opening, which tube has an ascending discharge leg, a U-bend (4) located below the rim of the container, a descending suction leg and the opening (5) of the suction leg is located below the bottom of the container, such that when liquid flows through the outlet the container will be emptied.
- 25 5. Device according to one of the preceding claims,  
c h a r a c t e r i z e d i n that the activated means are the moving means for the container and/or the attracting means, and that the attracting means in itself influences the beads proportionally with the distance between the beads and the attracting means.
- 30 6. Device according to one of the claims 1 - 4,  
c h a r a c t e r i z e d i n that the activating means consists of switches for supply energy and thereby activating the attracting means.

7. Device according to one of the claims 1 - 5,  
c h a r a c t e r i z e d i n that the particles are paramagnetic beads and the  
attracting means for the said particles are means for creation of a magnetic  
field, preferably permanent magnets.
- 5 8. Device according to one of the claims 1 - 6,  
c h a r a c t e r i z e d i n that the beads or particles are electrically charged  
and that the attracting means of the said particles are means for creation of an  
electric field.
9. Method for purifying organic material, preferable in a solution,  
10 c h a r a c t e r i z e d i n that it comprises the following steps;  
a) to add the organic material to be purified together with beads or  
particles for adsorption and/or binding of the organic material in a  
container (1) supplied with controllable inlet and outlet for liquids  
located at a distance from each other,  
15 b) to fill the container with a liquid,  
c) to activate means for attracting the beads or particles,  
d) to empty the liquid out of the container such that the beads or particles  
with the organic material adsorbed remain in the container.
10. Method according to claim 9 wherein the outlet (3) of the container is  
20 connected to a U-formed tube with the ascending leg on the outside of the  
container and the U-bend (4) defining the upper liquid level in the container  
below the rim of the container and the outlet (5) of the descending leg of the  
tube is located below the outlet of the container,  
c h a r a c t e r i z e d i n that in step (b) the container is filled with liquid  
25 to a level which is below the horizontal U-bend and in step (d) the container  
is filled to a level which is on or above the horizontal U-bend, such that the  
container will be emptied for the liquid.



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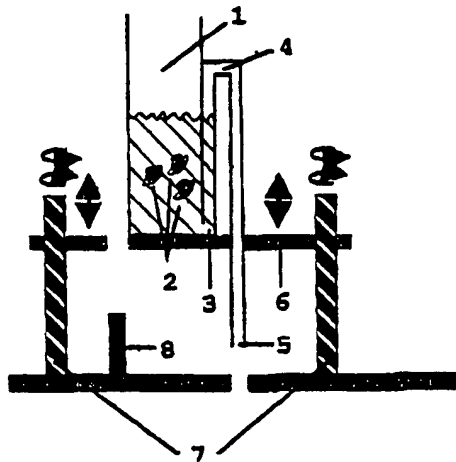


Fig. 1a

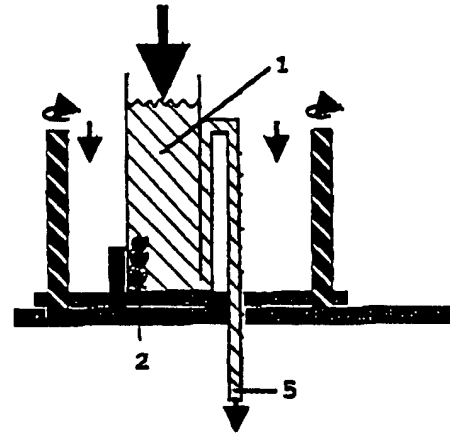


Fig. 1b

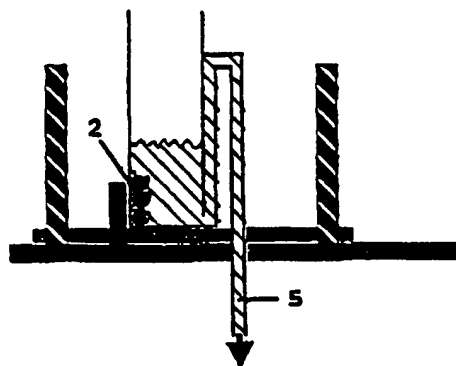


Fig. 1c

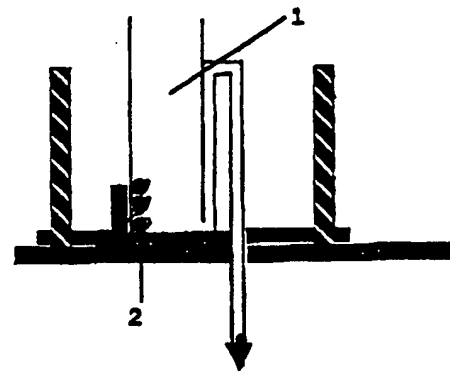


Fig. 1d

1  
INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 98/00271

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12M 1/00, C02F 1/28 // B03C 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

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IPC6: C02F, C12M, C12N, B03C

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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5622831 A (PAUL A. LIBERTI ET AL), 22 April 1997 (22.04.97), claims 3-4, abstract --	1-3,5-7,9
X	EP 0687505 A1 (BOEHRINGER MANNHEIM GMBH), 20 December 1995 (20.12.95), column 3, line 26 - line 42, abstract --	1-3,5-7,9
X	US 5439586 A (ADRIAN J. RICHARDS ET AL), 8 August 1995 (08.08.95), see esp. abstract, column 5, line 27 - line 52, claims --	1-3,5-7,9

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 83020405 A1 (REED, THOMAS, A.), 21 July 1983 (21.07.83), see abstract and figures  -- -----	1-3,5-7,9

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**INTERNATIONAL SEARCH REPORT**  
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03/11/98

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US 5622831 A	22/04/97	US 5541072 A	30/07/96
		US 5795470 A	18/08/98
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